REMARKS

Reconsideration of the application in view of the above amendments and following remarks is requested. Claims 1-29 are now in the case. Claims 11, 15 and 18 have been amended.

The specification was objected to and claims 1-29 were rejected under 35 U.S.C. § 112, first paragraph, on the grounds that the specification fails to provide an enabling disclosure. The Examiner believes that in view of the complex structure of fibrinogen (as taught by Mosher), a lack of evidence of production of biologically active fibrinogen, and the allegedly unpredictable nature of transgenic technology, the practitioner would not have accepted that the claimed animals would have been able to have generated biologically active fibrinogen. The Examiner further believes that the specification fails to provide any guidance in regard to how one would have recovered biologically active fibrinogen.

Applicants respectfully traverse this grounds of rejection. The Examiner's specific points will be addressed individually:

1. The specification fails to provide an enabling disclosure for the preparation of any and all transgenic animals. Applicants have prepared biologically active (clottable) fibrinogen in the milk of transgenic mice in accordance with the teachings of the specification. Evidence of such preparation is provided herewith in the Declaration of Gerald W. Lasser Under 37 C.F.R. § 1.132. As shown therein, fibrinogen in the milk of transgenic mice prepared as disclosed in Applicants' specification clotted in the presence of thrombin and calcium. Analysis of the clots showed that fibrinopeptides A and B had been removed from the α and β subunits, respectively, and that the clots had been cross-linked by factor XIII and by a transglutaminase present in the milk. Additional experimental evidence, described in the accompanying Declaration of Donna E. Prunkard Under 37 C.F.R. § 1.132,

demonstrates that the transgenic mouse milk contained fully assembled fibrinogen hexamer.

Applicants have provided detailed protocols for the production of transgenic fibrinogen in the milk of sheep. Given applicants' experience with transgenic mice, one skilled in the art would be able to carry out the procedures described for producing fibrinogen in sheep with a high level of confidence of success.

Applicants have further provided guidance for use of other host species, including pigs, goats, cattle and rats. In this regard, the Examiner is directed to Applicants' specification, for example at pages 11-17. Methods for producing transgenic mammals are known in the art, and are generally considered applicable to mammals in general. See, for example, the disclosure of Meade et al. (U.S. Patent 4,873,316, which is of record) at column 2, lines 57-58 ("Any mammal may be usefully employed in this invention."). Meade et al. further teach that cows, sheep, goats, mice, oxen, camels and pigs are preferred hosts. Drohan et al. (WO 92/11757; of record) teach methods for producing protein C in transgenic animals that are useful in "[a]ll lactating animals" (page 9 at lines 31-32). Pursel at page 1282 does not teach that production in particular species is not possible; Pursel merely addresses the inefficiency of certain methods in certain species. This inefficiency does not prohibit the use of these species (e.g. pigs) from being used as transgenic hosts, nor does it demonstrate that methods do not exist for obtaining suitable expression levels in a particular host. Hennighausen discloses that the use of intronic sequences, which Applicants teach to be desirable, may result in consistent, high-level expression. Similar teachings are provided by Archibald et al. (WO 90/05188; of record), who disclose that "high yields can be obtained using constructs comprising some but not all, of the naturally occurring introns in a gene" (page 5 at lines 30-32). Meade teaches (column 2, lines 61-68) that, although expression levels

may vary from animal to animal, selection of promoters and signal sequences for use in a particular animal may be done "easily" by one of skill in the art. Finally, applicants wish to direct the Examiner to Table 1 of Pursel, which discloses expression of transgenes not only in mammals, but also in chickens and fish. These disclosures, when taken as a whole, teach that all mammals are regarded as useful transgenic hosts and that methods of optimizing transgenic expression are known. The scope of Applicants' claims is thus well within what is regarded in the art as feasible.

2. The specification fails to provide an enabling disclosure for the preparation and use of any and all gene constructs comprising any fibrinogen gene. Applicants are somewhat puzzled by this objection in view of the Examiner's comment on page 9 of the Office Action that "the parameters for designing of appropriate constructs" were known in the art. Applicants agree that selection of secretion signals, promoters and other elements of the claimed gene constructs was and is a matter of routine design choice for those skilled in the art. The routine nature of the required experimentation is disclosed by Meade et al. at column 2, lines 66-68 ("one of skill in the art may easily make such choices"), and in column 3 at lines 1-30. The Examiner's attention is also directed to the disclosures of Drohan et al. and Archibald et al., which are discussed above, as well as to Applicants' specification, such as at pages 8-10.

Applicants' claims are limited to constructs that would be expected to perform the requisite function. Claims 1 and 20 require that each of the first, second and third DNA segments is operably linked to additional DNA segments required for its expression in a mammary gland of a host female mammal. For purposes of clarity, claim 11 has been amended to recite that each of the first, second and third gene fusions comprises a β-lactoglobulin promoter operably linked to the respective DNA segment.

With regard to Hennighausen, Applicants respectfully submit that the difficulties and limitations addressed therein are related to the economics of transgenic production in a limited set of host/vector systems, not to a lack of generally applicable methods ("a number of difficulties have to be overcome before this production system becomes economically effective"; page 7, right-hand column). The passage on page 4 referred to by the Examiner is directed to quantitative differences between species and particular constructs. Nonetheless, Hennighausen discloses in Table 1 nine different proteins that have been produced in the milk of transgenic animals. Methods for overcoming inconsistent expression of transgenes are discussed by Hennighausen on page 6, and include the inclusion of genomic sequences. Moreover, as disclosed by Hennighausen, low expression levels often arise from variation between lines of animals derived from different founder animals. It would be evident to one skilled in the art that it is desirable to screen animals and use only high producers in establishing transgenic herds. The disclosures of Drohan et al. and Archibald et al. demonstrate that methods for obtaining economically viable expression levels existed at the time of Applicants' invention, and further demonstrate that the limitations discussed by Hennighausen may be overcome without undue experimentation.

3. The specification fails to provide adequate guidance in regard to how to use any and all transgenic non-human mammalian embryos and mammals because the only animals for which guidance is offered are those that express biologically active fibrinogen in their mammary glands. Applicants respectfully traverse this grounds of rejection. Claims 1-19 and 25 as filed are limited to animals (or their progeny) that produce biologically active fibrinogen. More specifically, claim 1 recites "female progeny that . . . produce milk containing biocompetent fibrinogen encoded by said segments"; claim 11 recites

"said DNA segments are expressed in a mammary gland of said mammal or its female progeny and biocompetent fibrinogen is secreted"; claim 16 recites "fibrinogen encoded by said segments is secreted into milk of said mammal"; and claim 25 recites that the female mammal "produces milk containing biocompetent fibrinogen encoded by said DNA segments." The remaining claims include male animals that may not produce fibrinogen but are useful nonetheless, such as for breeding stock. The utility of such male animals was acknowledged by the Examiner at page 9 of the Office Action.

4. The specification fails to provide an enabling disclosure because no requirement is present in claim 15 that the egg and the female be of the same species. Claim 15 has been amended, thereby overcoming this objection and rejection.

5. The specification fails to provide an enabling disclosure for how to make and use any and all of the animals within the scope of the invention of claim 18. It is the Examiner's opinion that the teachings of the specification require that the DNA be integrated into the genome and be expressed. Applicants respectfully traverse this grounds for objection and rejection. As disclosed in the specification, for example on page 15, DNA is injected into a pronucleus of an egg, after which the pronuclei fuse. Embryos are inserted into the recipient females, and offspring are screened for the transgenes. The embryos recited in claim 18 are the products of the injection process, which process will, in general, produce a mixture of embryos including those that have integrated the transgenes and those that have not. Both groups of embryos are used (inserted into the recipient females) because screening for integration of the genes of interest does not occur until after birth of the offspring. The entire class of embryos developing from the microinjected eggs are useful intermediates in the production of transgenic animals and are fully enabled by Applicants' specification.

The Examiner questioned how one would "use an animal that did not express said DNA". However, claim 18 is directed to embryos, which need not express the DNA to be useful (and in all liklihood would not express the DNA in the embryonic state).

In addition, the Examiner indicated that the specification fails to provide enablement for recovering biologically active fibrinogen. Applicants wish to direct the Examiner's attention to the specification at page 17, wherein the use of such standard practices as skimming, precipitation, filtration and chromatography is disclosed. Such techniques are routinely used for protein purification. See, e.g., Stinnakre et al. (Animal Biotechnology 3:245-255, 1992; copy enclosed), Farrell et al. (J. Biol. Chem. 269:226-231, 1984; of record), and Matthias et al. (Thrombosis Res. 7:861-870, 1975; copy enclosed). It is generally believed that milk does not present any extraordinary difficulties for protein purification (Hennighausen, last paragraph on page 7; Stinnakre et al., final sentence in Summary).

Claims 1-15 and 18-25 were rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. More specifically, the Examiner believes that claims 1 and 11 are vague and indefinite because it is unclear to the Examiner what is required for operative linkage between the secretion signal and the fibrinogen chains. Claims 1 and 20 are believed to be unclear because no indication of the operation required for the link to the cited additional DNA segments is present, the the phrase "required for its expression" is unclear. Claims 1 and 20 are also believed to be unclear in the recitation of "its expression". Claim 5 is believed to be vague and indefinite as to the nature of the operative linkage between the segments and the promoter. Claim 18 is believed to be inaccurate in the recitation of "embryo" and the singular "nucleus". Claim

19 is believed to be vague and indefinite in the recitation of "recoverable".

This rejection is traversed in part and overcome in part. The term "operably linked" is used within the claims for its art-recognized meaning: the recited elements are joined so that they function for their intended purposes. A similar term ("operatively linked") is used by Meade et al. at column 1 and in claim 1. One of ordinary skill in the art would recognize, for example, that a secretion signal, when operably linked to a polypeptide, directs the secretion of the polypeptide from the cell that produces it (as disclosed in Applicants' specification on, e.g., page 10 at lines 17-19); and that a trascription promoter, when operably linked to a coding sequence, provides for the transcription of that sequence. The construction of expression cassettes is routine in the art. "Additional DNA segments required" for expression of coding segments would be understood by those skilled in the art to be promoter sequences and sequences which provide for termination of transcription and polyadenylation of mRNA, as taught in the specification at page 10. With regard to the recitation of "its expression", Applicants submit that the usage is grammatically correct and unambiguous in view of the use of the singular "each" as the subject of the clause in question. With regard to the recitation of "nucleus" in claim 18, Applicants have amended the claim to recite "nuclei". It will be understood that not <u>all</u> nuclei must contain the transgenes as disclosed in the specification at pages 15-16.

"Recoverable" is used for its art-recognized meaning to indicate that the protein is produced at higher than trace levels that can be detected by sophisticated analytical techniques but that cannot otherwise by separated from the milieu in which they are produced.

Claims 1-8 and 11-29 were rejected under 35 U.S.C. § 103 as being unpatentable over Meade et al., Archibald et al., and Roy et al. In summary, the Examiner

believes that the claimed invention would have been *prima facie* obvious to one of ordinary skill in the art because the use of transgenic animals as bioreactors is taught by Meade et al. and Archibald et al., and Roy et al. teach the production of recombinant human fibrinogen in eukaryotic cells.

Applicants respectfully traverse this grounds of rejection. As noted by the Examiner on pages 2-3 of the instant Office Action, fibrinogen is a structurally complex molecule requiring extensive processing and assembly for biological activity, making Applicants' invention a significant and unexpected achievement. Biologically active fibrinogen is a hexameric molecule, making its production in transgenic animals unprecedented. Given the results of the experiments of Roy et al. and others using cultured eukaryotic cells, on could not have predicted Applicants success in producing useful amounts of biologically active, transgenic fibrinogen.

Roy et al. disclose the production of about 2 μg of fibrinogen per 2 x 10^6 cells per 24 hours. Although Roy et al. are silent as to the concentration of the fibrinogen produced, even assuming a culture volume as small as 1 ml gives an expression level of only 2 μg/ml of culture media per day (comparable to the expression seen in BHK cells by Farrell et al., Blood 74:55a, 1989; and Farrell et al., Biochemistry 30:9414-9420, 1991, copies of which are enclosed)[1]. Prunkard et al. (XIV Congress of the International Society on Thrombosis and Haemostasis, 1993; cited on page 2 of Applicants' specification; copy enclosed herewith) attempted to increase expression of recombinant fibrinogen in baby hamster kidney (BHK) cells by amplification. Prunkard et al. increased gene copy number and amplified the level of fibrinogen mRNA, but no increase in correctly processed fibrinogen resulted. These results

[1]Roy et al. report that expression levels seen in COS cells were comparable to production of fibrinogen by cultured HepG2 cells. Farrell et al. (1991) report (p. 9416, right column) that HepG2 cells produced 1.3 μg/ml/day.

confirm the complexity of fibrinogen maturation and processing, and suggest (see Prunkard et al.) the need for some liver-specific pathway(s) to produce biologically active fibrinogen in significant quantities.

In contrast to the cell culture system disclosed by Roy et al., Applicants' transgenic animals have produced as much as 1000 mg of fibrinogen per liter of milk as shown in the accompanying Declaration of Donna E. Prunkard Under 37 C.F.R. § 1.132. Furthermore, the percentage of correctly assembled fibrinogen in the milk of transgenic mice actually increased with higher levels of fibrinogen protein. The results disclosed by Prunkard et al. using BHK cells would suggest to one skilled in the art that increasing fibrinogen gene expression in non-hepatic cells would not result in increased production of assembled fibrinogen hexamer.

Given the low level of expression observed by Roy et al. and the unsuccessful amplification attempts of Prunkard et al., one could not have predicted useful levels of fibrinogen production in the milk of transgenic animals. There would, therefore, have been no motivation to produce such transgenic animals, without a reasonable expectation that useful results would have been obtained (particularly in view of the costs involved--see Hennighausen at page 4), even assuming, *arguendo*, that mammary epithelial cells could produce any amount of biocompetent fibrinogen.

Meade et al. and Archibald et al. also fail to teach or suggest the production of biocompetent fibrinogen in transgenic animals.

Claims 9 and 10 were rejected under 35 U.S.C. § 103 as being unpatentable over Meade et al., Archibald et al., and Roy et al., and further in view of Chung et al. and Lewin. Meade et al., Archibald et al., and Roy et al. were cited as above. Chung et al. was cited as teaching the sequence of human Bβ-fibrinogen. Lewin was cited as teaching the need for a Met initiation codon.

Applicants respectfully traverse this grounds of rejection. The Meade, Archibald, and Roy references have been discussed above. Briefly, the complexity of fibrinogen and the low levels of expression seen by Roy et al. make Applicants' results unexpected and would not have motivated the ordinarily skilled practitioner to attempt to produce fibrinogen in the milk of transgenic animals. The Chung et al. and Lewin references do not remedy the deficiencies of the primary references.

On the basis of the above amendments and remarks, Applicants believe that each rejection has been addressed and overcome. Reconsideration of the application and its allowance are requested. If for any reason the Examiner feels that a telephone conference would expedite prosecution of the application, the Examiner is invited to telephone the undersigned at (206) 442-6673.

Respectfully Submitted,
Ian Garner et al.

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Enclosures:
Amendment Fee Transmittal (in duplicate)
Declaration of Gerald W. Lasser
Declaration of Donna E. Prunkard
5 References
Postcard